

VU Research Portal

A diet rich in unsaturated fatty acids prevents progression toward heart failure in a rabbit model of pressure and volume overload

den Ruijter, H.M.; Verkerk, A.O.; Schumacher, C.A.; Houten, S.M.; Belterman, C.N.; Baartscheer, A.; Brouwer, I.A.; Van Bilsen, M.; de Roos, de, B.; Coronel, R.

published in

Circulation. Heart Failure
2012

DOI (link to publisher)

[10.1161/CIRCHEARTFAILURE.111.963116](https://doi.org/10.1161/CIRCHEARTFAILURE.111.963116)

document version

Publisher's PDF, also known as Version of record

[Link to publication in VU Research Portal](#)

citation for published version (APA)

den Ruijter, H. M., Verkerk, A. O., Schumacher, C. A., Houten, S. M., Belterman, C. N., Baartscheer, A., Brouwer, I. A., Van Bilsen, M., de Roos, de, B., & Coronel, R. (2012). A diet rich in unsaturated fatty acids prevents progression toward heart failure in a rabbit model of pressure and volume overload. *Circulation. Heart Failure*, 5(3), 376-384. <https://doi.org/10.1161/CIRCHEARTFAILURE.111.963116>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:

vuresearchportal.ub@vu.nl

A Diet Rich in Unsaturated Fatty Acids Prevents Progression Toward Heart Failure in a Rabbit Model of Pressure and Volume Overload

Hester M. Den Ruijter, PhD; Arie O. Verkerk, PhD; Cees A. Schumacher, RA;
Sander M. Houten, PhD; Charly N.W. Belterman, RA; Antonius Baartscheer, PhD;
Ingeborg A. Brouwer, PhD; Marc van Bilsen, PhD; Baukje de Roos, PhD; Ruben Coronel, MD, PhD

Background—During heart failure (HF), cardiac metabolic substrate preference changes from fatty acid (FA) toward glucose oxidation. This change may cause progression toward heart failure. We hypothesize that a diet rich in FAs may prevent this process, and that dietary ω 3-FAs have an added antiarrhythmic effect based on action potential (AP) shortening in animals with HF.

Methods and Results—Rabbits were fed a diet containing 1.25% (w/w) high oleic sunflower oil (HF- ω 9, N=11), 1.25% fish oil (HF- ω 3, N=11), or no supplement (HF-control, N=8). Subsequently, HF was induced by volume and pressure overload. After 4 months, HF-parameters were assessed, electrocardiograms were recorded, and blood and ventricular tissue were collected. Myocytes were isolated for patch clamp or intracellular Ca^{2+} -recordings to study electrophysiologic remodeling and arrhythmogenesis. Both the HF- ω 9 and the HF- ω 3 groups had larger myocardial FA oxidation capacity than HF control. The HF- ω 3 group had significantly lower mean (\pm SEM) relative heart and lung weight (3.3 ± 0.13 and 3.2 ± 0.12 g kg^{-1} , respectively) than HF control (4.8 ± 0.30 and 4.5 ± 0.23), and shorter QTc intervals (167 ± 2.6 versus 182 ± 6.4). The HF- ω 9 also displayed a significantly reduced relative heart weight (3.6 ± 0.26), but had similar QTc (179 ± 4.3) compared with HF control. AP duration in the HF- ω 3 group was $\approx 20\%$ shorter due to increased I_{to1} and I_{K1} and triggered activity, and Ca^{2+} -aftertransients were less than in the HF- ω 9 group.

Conclusions—Dietary unsaturated FAs started prior to induction of HF prevent hypertrophy and HF. In addition, fish oil FAs prevent HF-induced electrophysiologic remodeling and arrhythmias. (*Circ Heart Fail.* 2012;5:376-384.)

Key Words: heart failure ■ nutrition ■ lipids ■ remodeling heart failure ■ electrophysiology

In heart failure (HF), metabolic substrate preference shifts from fatty acid oxidation (FAO) toward glucose oxidation.¹ It has been suggested that this change underlies the progression toward HF.² As a consequence, driving the balance toward FAO is expected to prevent or slow down the progression of HF and reduce the associated arrhythmias. We hypothesize that this can be accomplished by a diet rich in fatty acids (FAs).

Clinical Perspective on p 384

Heart failure is associated with a prolongation of ventricular action potential (AP), which plays an important role in the genesis of life-threatening ventricular tachyarrhythmias.³ Dietary ω 3-FAs cause a shortening of the AP duration (APD)

in healthy animals.⁴ Acute application of ω 3-FAs to isolated myocytes from hearts of patients with HF led to APD shortening and reduced incidence of triggered activity.⁵ It is not known whether dietary ω 3-FAs exert the same action in vivo.

The Diet and Reinfarction and GISSI trials (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico) addressed the potential beneficial effects of the Mediterranean diet and showed that increased intake of fish oil reduced cardiovascular death following a myocardial infarction.^{6,7} This mainly was attributed to risk reductions in sudden death in the GISSI Trial.⁷ Because ventricular tachyarrhythmias often precede sudden death, the effect of fish oil on arrhythmogenesis has been studied intensively in

Received May 30, 2011; accepted March 26, 2012.

From the Experimental Cardiology Group of the Heart Center (H.M.D.R., C.A.S., C.N.W.B., A.B., R.C.) and the Department of Anatomy, Embryology and Physiology (A.O.V.), Laboratory Genetic Metabolic Diseases (S.M.H.), Academic Medical Center; Department of Health Sciences and the EMGO Institute for Health Care Research, VU University Amsterdam (I.A.B.); Department of Physiology, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands (M.V.B.); and the Rowett Institute of Nutrition and Health, University of Aberdeen, UK (B. de R.).

Drs Den Ruijter and Verkerk contributed equally to this work.

The online-only Data Supplement is available at <http://circheartfailure.ahajournals.org/lookup/suppl/doi:10.1161/CIRCHEARTFAILURE.111.963116/-/DC1>.

Correspondence to Ruben Coronel, Experimental Cardiology Group, Academic Medical Center, Room K2-112, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, E-mail rubencoronel@gmail.com

© 2012 American Heart Association, Inc.

Circ Heart Fail is available at <http://circheartfailure.ahajournals.org>

DOI: 10.1161/CIRCHEARTFAILURE.111.963116

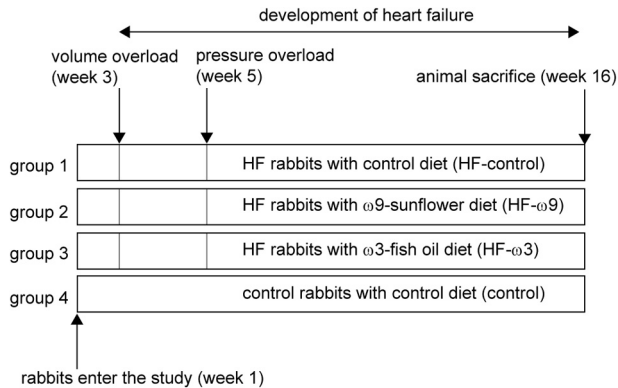


Figure 1. Study outline.

humans and animals.^{5,8–10} These studies show reductions in severity and number of arrhythmias by fish oil. However, the GISSI-HF trial showed that fish oil supplementation for 4 years in patients with severe HF resulted in a relatively small absolute risk reduction in total mortality compared with the original GISSI trial.¹¹

The difference between the 2 GISSI trials^{7,11} is that in the latter fish oil was provided to patients that had already developed severe HF,¹¹ whereas in the first GISSI trial, patients with a recent myocardial infarction and with developing hypertrophy were included.⁷ The different outcomes between trials may be explained by a diet-induced retardation of the progression of HF in the first but not the second GISSI trial.

We tested whether long term supplementation of the diet with unsaturated (ω 3- or ω 9-) FAs prior to the development of HF by combined volume and pressure overload in rabbits attenuates structural and functional remodeling and leads to less hypertrophy and arrhythmias. Rabbits without dietary supplements were used as control. We measured parameters of HF, electrocardiograms, and markers of FAO, as well as triggered arrhythmias and cardiac electrophysiological parameters 4 months after the induction of HF.

Methods

Design of the Study

The investigation was approved by the local ethical committee and conformed to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1996).

The design of the study is shown in Figure 1. Male New Zealand White rabbits (2.5–3 kg) were allocated to 1 of 4 groups. Group 1 consisted of rabbits on a diet (150 g/d) without added FAs that underwent volume and pressure overload (HF control; $n=8$). Group 2 and 3 consisted of rabbits on a diet (150 g/d) supplemented with either 1.25% (w/w) ω 9-sunflower oil (HF- ω 9; $n=11$) or ω 3-fish oil (HF- ω 3; $n=11$) that underwent volume and pressure overload. Group 4 consisted of rabbits on a diet (150 g/d) without added FAs (control; $n=5$).

Heart failure was induced by combined volume and pressure overload as described before, typically inducing concentric hypertrophy as well as some left ventricular dilatation of the heart.¹² Volume overload was induced by aortic valve rupture (100% pulse pressure increase). Three weeks later, pressure overload was created by a \approx 50% suprarenal aortic stenosis. After 4 months, the rabbits were anesthetized by a combination of ketamine (50 mg im) and xylazine (10 mg im), blood was collected, and an ECG was recorded. The rabbits were then heparinized (5000 IU) and killed by intravenous injection of pentobarbital (240 mg). Body weight, heart weight,

and lung weight were measured, and the presence of ascites and pericardial and thoracic fluid documented.¹⁰ Left ventricular tissue was collected and stored at -80°C .

Diets

The diets (RDS, Wijk bij Duurstede, NL) contained \approx 3.5% (w/w) fat, mainly linoleic acid. The addition of 1.25% sunflower oil and 1.25% ω 3-fish oil increased mono-unsaturated FAs in the ω 9-diet (C18:1 ω 9), and eicosapentaenoic acid and docosahexaenoic acid (EPA-C20:5 ω 3 and DHA-C22:6 ω 3) in the ω 3-diet (see online-only Data Supplement Table I).

Analysis of Diet, Tissue, and Plasma

Lipids from the food and tissue were extracted according to Folch et al.¹³ Phospholipids were isolated with Bond Elut Aminopropyl solid phase extraction columns. Saponification and methylation of the phospholipids with boron trifluoride (Pierce) was performed, and the formed FA methyl esters were subjected to capillary gas chromatography using a Chrompack column (Fused Silica, Chrompack), a flame ionization detector, and H_2 as carrier gas. FA methyl esters were expressed as a fraction of the total amount. To determine the plasma lipid profiles (total, low density lipoprotein [LDL], and high density lipoprotein [HDL] cholesterol, and triglycerides), samples were analyzed with spectrophotometry (enzymatic color test) using commercial kits (Roche Diagnostics GmbH).

Cell Preparation

Midmyocardial myocytes were isolated by enzymatic dissociation from the left ventricular free wall.¹⁴ Aliquots of cells were superfused (37°C) in a recording chamber on the stage of an inverted microscope. Quiescent rod-shaped cross-striated cells with a smooth surface were selected.

Electrophysiology

Electrocardiogram

QT segments were corrected for heart rate using linear regression analysis obtained from 37 healthy control rabbits. Accordingly, the QT intervals were normalized to an RR interval of 4 Hz using the formula $\text{QTc} = \text{QT} - 0.26 \cdot (\text{RR} - 250)$.

Current-Clamp and Voltage-Clamp

See the online-only Data Supplement.

Ca^{2+} Transients

Intracellular Ca^{2+} (Ca^{2+}_i) was measured in indo-1-am loaded myocytes as described previously.¹² Dual wavelength emission of indo-1 was recorded ([405–440]/[505–540] nm, excitation at 340 nm), and free Ca^{2+}_i was calculated.¹²

Gene Expression and Enzymatic Activity Determination

Left ventricular samples were homogenized and total RNA was isolated. An additional wash step of 70% ethanol increased the RNA purity. Integrity of the RNA was checked by means of 260/280 ratio. cDNA synthesis and quantitative polymerase chain reaction (qPCR) assays were performed (for primers see online-only Data Supplement Table II).¹⁵ Results were normalized to the geometric mean of 2 reference genes, that is, cyclophilin A and hypoxanthine guanine phosphoribosyl transferase,¹⁶ using qBase software.¹⁷

Activity

Left ventricular tissue was homogenized in cold phosphate-buffered saline followed by sonication on ice (twice 40J at \approx 8W output). Homogenates were centrifuged for 10 minutes at 1000 g. Carnitine palmitoyltransferase 1 (CPT 1) activity was assessed by measuring the [$\text{U}-^{13}\text{C}$] palmitoylcarnitine from carnitine, and [$\text{U}-^{13}\text{C}$] palmitoyl-CoA on the tandem-mass spectrometer as previously described.¹⁸ Incubations were performed in the presence and absence of 0.2 mmol/L malonyl-CoA. The activity inhibited by malonyl-CoA was considered to represent CPT 1.

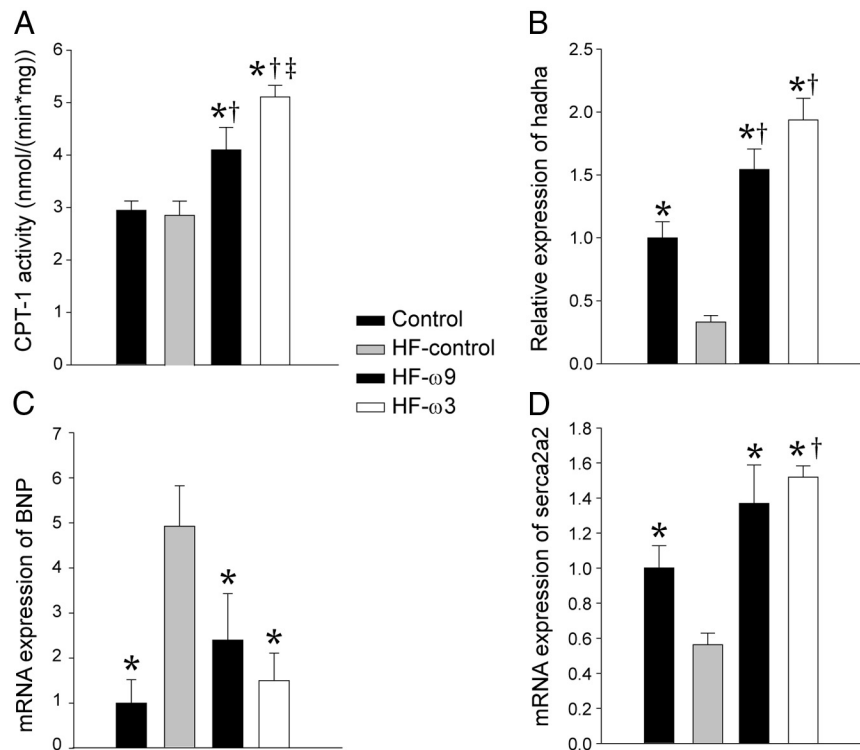


Figure 2. Fatty acid oxidation capacity. **A**, Carnitine palmitoyltransferase 1 activity in the ventricular myocardium of the 4 groups. Data are obtained from 5 to 6 animals per group. **B**, mRNA expression of hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), α subunit relative to control. Data are obtained from at least 3 animals per group. **C**, Myocardial mRNA levels of markers of hypertrophy, pro-BNP. **D**, SERCA2a. All data are obtained from at least 5 rabbits per group and are relative to control. * $P < 0.05$ versus HF control; † $P < 0.05$ versus control; ‡ $P < 0.05$ versus HF- ω 9 (1-way ANOVA).

Proteomics

Cardiac tissue protein homogenates were prepared by grinding frozen tissue from 5 HF control, 8 HF- ω 9, and 10 HF- ω 3 rabbits in liquid nitrogen and homogenizing them in a lysis buffer (7 mol/L Urea, 2 mol/L Thiourea, 2% CHAPS [3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonic acid], 0.06% proteinase inhibitor cocktail [Roche]). Protein concentrations were determined using the RC/DC [reducing agent compatible, as well as detergent compatible] assay (BioRad), and 300 μ g of protein were loaded per gel. BioRad IPG strips (pI 5–8) were used for the separation of proteins in the first dimension, and SDS-PAGE was performed on 18x18 cm acrylamide gradient (8%–16%) gels for the separation of proteins in the second dimension. Flamingo (Biorad) fluorescent-stained gels were analyzed using SameSpots (Nonlinear Dynamics). Spot density values were exported from the SameSpots software into R 2.11.1 (R Foundation for Statistical Computing) to perform statistical analysis as described below. Spots were excised from the gel using a robotic spot cutter (BioRad), trypsinized using a MassPrep Station (Micromass), and analyzed by LC-ESI-MS/MS.¹⁹

Statistics

Data are mean \pm SEM (n,N: number of cells, rabbits). Data were statistically analyzed using 1- or 2-way ANOVA, on ranks (with Kruskal-Wallis test) or repeated measures ANOVA if appropriate. In repeated measures ANOVA, voltage steps were modeled as a categorical factor and cells as the repeated factor, without interaction. Post hoc testing for multiple comparisons was done with the Holm-Sidak, Fisher LSD Method (with parametric ANOVA), or Dunn test (with nonparametric ANOVA); $P < 0.05$ was considered statistically significant).

Results

Fatty Acid Oxidation

Left ventricular activity of CPT 1, a rate-limiting enzyme controlling mitochondrial import of FAs,²⁰ was increased significantly in the HF- ω 9 and HF- ω 3 groups compared with HF control and control (Figure 2A). The cardiac mRNA level

of the α -subunit of mitochondrial trifunctional protein (HADHA), another FAO enzyme, was markedly decreased in the HF control group compared with healthy control rabbits. The ω 9- and ω 3-FA-enriched diets prevented the HF-mediated decline in HADHA mRNA levels and even resulted in significantly higher levels than in healthy control rabbits (Figure 2B, $P < 0.05$ nonparametric ANOVA followed by Dunn test). Glutamate dehydrogenase was not different excluding a general proliferation or enlargement of the mitochondria in these groups (data not shown).

Plasma and Tissue Content

The diets rich in ω 3-FAs resulted in a significant reduction of plasma triglycerides compared with HF control and HF- ω 9. Triglyceride content in the ω 3-group was not different from that of control rabbits (Table). There were no differences in total cholesterol or LDL- or HDL-cholesterol between the groups (data not shown).

In the HF- ω 3 group, the ω 3-FA tissue-content comprised $15.5 \pm 0.5\%$ of the total FAs extracted compared with $5.3 \pm 0.3\%$ in the HF control group and $4.7 \pm 0.2\%$ in the HF- ω 9 group. The incorporation of ω 3-FAs was at the expense of ω 6-FAs and ω 9-FAs. There were no statistical differences in fat composition in the heart between the HF- ω 9 and the HF-control groups. Supplemental Table III shows the myocardial phospholipid fatty acid composition in the 4 groups (see online-only Data Supplement). The percentage of ω 9-FA oleic acid in the myocardial phospholipids is highest in the HF- ω 9 group and in the control group, and lowest in the HF- ω 3 group.

Unsaturated Fatty Acids Prevent Cardiac Hypertrophy During Developing Heart Failure

Weight gain was similar in the 4 groups (Table). The increase of the aortic pulse pressure immediately after aortic valve

Table. Characteristics (End of the Study)

	Control (N=5)	HF-Control (N=8)	HF- ω 9 (N=11)	HF- ω 3 (N=11)
Body weight (g)	3410 (80)	3430 (67)	3470 (44)	3590 (41)
Heart weight (g)	7.8 (0.37)*	16.7 (1.29)	12.7 (1.04)*†	11.7 (0.84)*†
Relative heart weight (g kg ⁻¹)	2.3 (0.10)*	4.8 (0.3)	3.6 (0.26)*†	3.3 (0.13)*†
Lung weight (g)	11.6 (0.94)	15.5 (1.05)	13.0 (1.49)	11.6 (1.25)
Relative lung weight (g kg ⁻¹)	3.4 (0.28)*	4.5 (0.23)	3.7 (0.39)	3.2 (0.12)*
Presence of ascites (%)	0	75	36	18
Presence of thoracic fluid (%)	0	88	18	9
Presence of pericardial fluid (%)	0	63	64	36
Plasma lipids	(N=5)	(N=4)	(N=5)	(N=5)
Triglycerides (mmol/L)	0.55 (0.03)*	0.90 (0.06)	0.61 (0.03)*	0.45 (0.05)*‡
Total cholesterol (mmol/L)	0.51 (0.07)	0.49 (0.10)	0.43 (0.02)	0.43 (0.02)
ECG parameters	(N=5)	(N=8)	(N=11)	(N=11)
QRS duration (ms)	48 (1.1)*	61 (0.9)	55 (1.1)*†	55 (0.9)*†
Heart rate (Hz)	2.8 (0.18)	3.1 (0.15)	3.0 (0.16)	2.8 (0.11)
QTc (ms)	143 (3.0)	161 (10.4)	155 (5.8)	137 (2.5)*‡

Data are presented as mean (SEM). Statistical significance was tested with ANOVA. * $P < 0.05$ vs HF-control; † $P < 0.05$ vs control; ‡ $P < 0.05$ vs HF- ω 9.

HF indicates heart failure.

rupture was 102 ± 1.3 , 101 ± 0.9 , and $100 \pm 0.7\%$ for HF control, HF- ω 9, and HF- ω 3, respectively ($P > 0.05$).

The HF control group had a significantly larger (relative) heart and lung weight than the control rabbits. In addition, the majority of the HF control rabbits had ascites and thoracic and pericardial fluid (Table). Although the trigger for the development of hypertrophy and HF was the same in all HF groups, the animals that received diets rich in ω 9- and ω 3-FAs had a significantly reduced relative heart weight compared with HF control ($P < 0.05$, ANOVA followed by Fisher least significant difference (LSD) test; Table). In addition, relative lung weight was lower in the HF- ω 3 group than HF control and did not differ from the control rabbits ($P < 0.05$, ANOVA followed by Fisher LSD test; Table). Supplemental Table IV shows the echocardiographic data that reflect the heart weight data (see online-only Data Supplement).

Pro-brain natriuretic peptide mRNA levels were measured as an indicator of cardiac hypertrophy. Relative myocardial mRNA levels of BNP were significantly lower in the HF- ω 3, HF- ω 9, and control group than HF control ($P < 0.05$, non-parametric ANOVA followed by Dunn test; Figure 2C).

In the HF- ω 3 and HF- ω 9 group, SERCa2a mRNA levels were significantly higher than HF control ($P < 0.05$, non-parametric ANOVA followed by Dunn test; Figure 2D). In the HF- ω 3 group, SERCa2a mRNA levels were even higher than in healthy control rabbits.

Fish Oil Fatty Acids Prevent Electrophysiological Remodeling

In line with the increased heart weight in the HF control group, the QRS duration was longer in HF control than control ($P < 0.05$, ANOVA followed by Fisher LSD test).²¹ The HF- ω 9 and HF- ω 3 groups also had longer QRS durations

than the control rabbits, but this was significantly less than the HF control group.

QTc was shorter in the HF- ω 3 rabbits than HF-control and HF- ω 9, and similar to that of healthy controls (Table). The heart rate did not differ between the groups. Figure 3A displays typical APs recorded from isolated myocytes of the 4 groups at 1 Hz pacing frequency. AP duration at 90% repolarization (APD₉₀) is prolonged significantly in the HF control group compared with control at pacing frequencies below 3 Hz (Figure 3B). Whereas APD₉₀ in the HF- ω 9 group did not differ from HF-control, it was significantly shorter in the HF- ω 3 group than in the HF control group and not different from control (Figure 3B). Similarly, the APD at 50% repolarization (APD₅₀, 2 Hz) in HF control and HF- ω 9 group was significantly longer (202 ± 8 and 197 ± 16 ms, respectively) than control (174 ± 6 ms). The APD₅₀ in the HF- ω 3 group (172 ± 10 ms) was similar to that of healthy control rabbits. Both HF- ω 3 and control were significantly different from HF control and HF- ω 9 (all $P < 0.05$, repeated measures ANOVA). Cell capacitance data reflected heart weight (see online-only Data Supplement Table V).

Figure 3C shows representative examples of Ca²⁺ transients in 2 Hz stimulated myocytes of all groups. Myocytes of the HF-control group have increased diastolic [Ca²⁺]_i, decreased Ca²⁺ transient amplitude, and slowed relaxation compared with control and in HF- ω 3 groups. In the HF- ω 9 group, diastolic Ca²⁺ was not different from the HF group, and thus increased compared with control. In the same HF- ω 9 group, however, Ca²⁺ transient amplitude and relaxation time were not different compared with control and HF- ω 3 groups. Data are summarized in Figure 3D. Diastolic Ca²⁺ levels were significantly lower in the HF- ω 3 (98 ± 7.9 nmol/L, $n = 19$, $P < 0.05$ ANOVA followed by Holm-Sidak test) than in HF control (128 ± 8.4 nmol/L, $n = 26$) and HF- ω 9 (120 ± 6.8

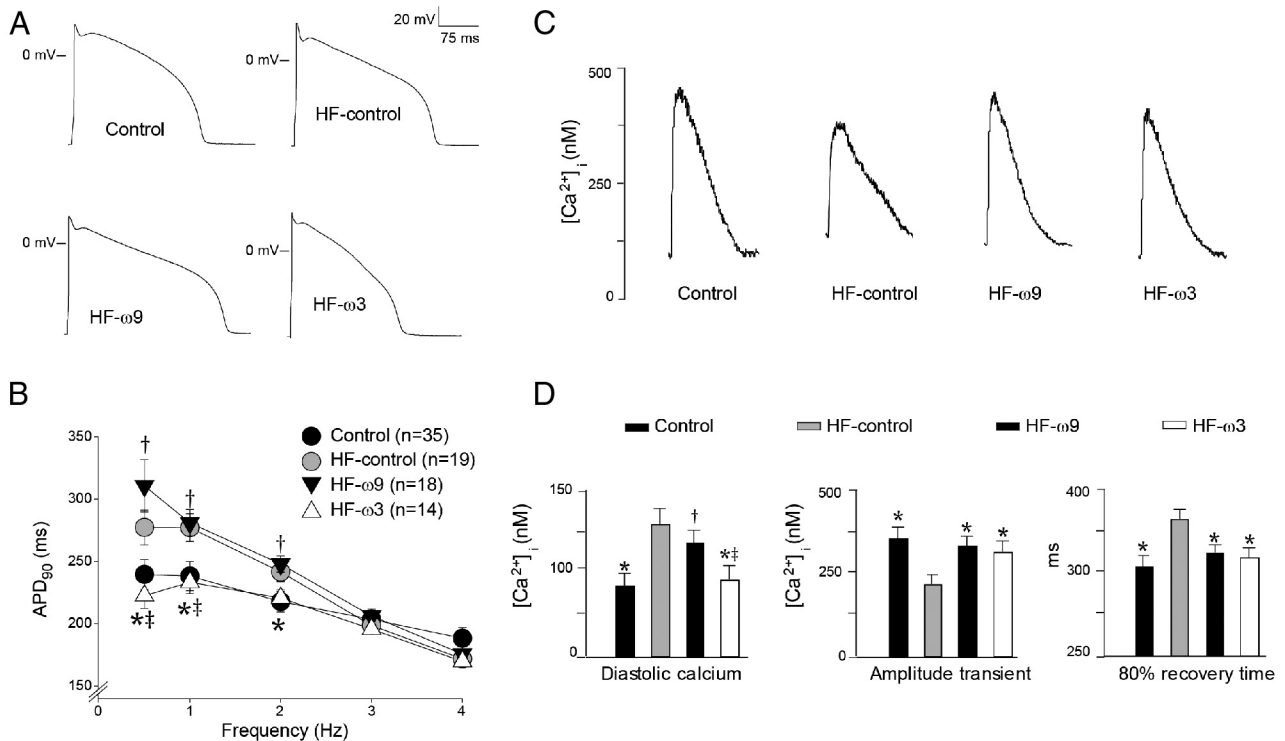


Figure 3. Fish oil prevents action potential (AP) prolongation. **A**, Representative APs (1 Hz pacing frequency). **B**, Averaged APD₉₀ as a function of pacing frequency. * $P < 0.05$ versus HF control; † $P < 0.05$ versus control; ‡ $P < 0.05$ versus HF-ω9 (repeated measures ANOVA). **C**, Representative examples of Ca²⁺ transient in 2-Hz stimulated myocytes. **D**, Average data of diastolic Ca²⁺, Ca²⁺ transients amplitude, and 80% recovery time of the Ca²⁺ transients in myocytes from the HF control (n=26 cells), HF-ω9 (n=32), HF-ω3 (n=19), and control (n=18) groups. * $P < 0.05$ versus HF control; † $P < 0.05$ versus control; ‡ $P < 0.05$ versus HF-ω9 (1-way ANOVA).

nmol/L, n=32). Diastolic Ca²⁺ levels were similar to those of healthy control rabbits (94±5.7 nmol/L, n=18).

Dietary Fish Oil Increases I_{to1} and I_{K1} in Heart Failure

To investigate the mechanism by which dietary fish oil prevents AP prolongation, we studied the major ion currents in the 4 groups (Figure 4A).

L-Type Ca²⁺ Current

Figure 4B shows typical examples and current-voltage (I-V) relationships of the L-type Ca²⁺ current (I_{Ca,L}). Mean I_{Ca,L} densities were similar in the control, HF control, and HF-ω3 group (Figure 4B). In the HF-ω9 group, I_{Ca,L} was significantly larger at plateau potentials (−5 to +5 mV) compared with all other groups ($P < 0.05$ repeated measures ANOVA). Voltage-dependency of I_{Ca,L} (in)activation did not differ significantly between the 4 groups (data not shown).

Na⁺-Ca²⁺ Exchange Current

Figure 4C shows typical examples and I-V relationships of the Na⁺-Ca²⁺ exchange current (I_{NCX}). There were no differences in the reverse (outward) mode and in the forward (inward) mode of the I_{NCX} between the 4 groups (Figure 4C).

K⁺ Currents

Figure 5A shows typical examples and I-V relationships of the transient outward K⁺ current (I_{to1}). Mean I_{to1} densities were significantly larger in the HF-ω3 group than HF control ($P < 0.05$ at +20 and +30 mV, repeated measures ANOVA; Figure 5A). In the HF-ω9 group, I_{to1} was larger at +30 mV

than HF control. Voltage dependency of I_{to} activation and inactivation did not differ significantly between the 4 groups (data not shown). Figure 5B shows typical examples and I-V relationships of the inward rectifier K⁺ current (I_{K1}). Mean I_{K1} densities were similar in the HF control group compared with control and were significantly larger at −110 and −100 mV in the HF-ω3 group than in the HF-ω9 group ($P < 0.05$ repeated measures ANOVA; Figure 5B). In addition, at −100 and −90 mV, I_{K1} densities of the HF control and HF-ω9 group were significantly smaller than control. Figure 5C shows typical examples and I-V relationships of the delayed rectifier K⁺ current (I_{Kr}). Neither the I_{Kr} tail densities nor the activation properties differed significantly between the 4 groups.

Fish Oil Inhibits Triggered Arrhythmias

Representative examples of transmembrane potentials in the 4 groups in the presence of 1 μmol/L noradrenalin after rapid pacing are shown in Figure 6A. In the HF control and the HF-ω9 myocytes, the last stimulated AP (arrow) is followed by delayed after depolarizations (DADs) and a triggered AP. In the HF-ω3 myocyte, a DAD was present, but triggered APs did not occur. In isolated myocyte of the control group no arrhythmias occurred.

Triggered APs and DADs were abundantly present in the HF control and in the HF-ω9 group compared with control (both $P < 0.05$, nonparametric ANOVA followed by Dunn test; Figure 6B). There was a significant reduction in both triggered APs and DADs in the HF-ω3 group compared with

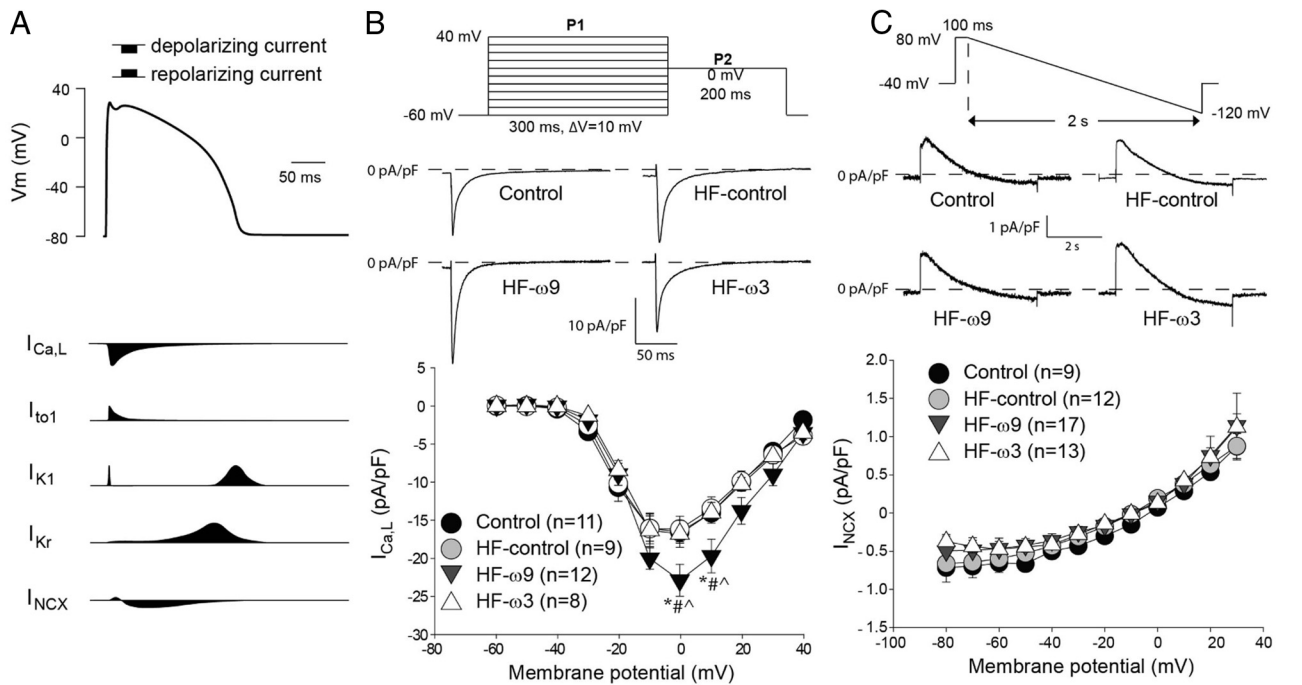


Figure 4. **A**, Schematic representation of the action potential and its major ion currents. **B**, Top: 2-step protocol for L-type Ca^{2+} current ($I_{Ca,L}$) measurement. Middle: typical examples of $I_{Ca,L}$ during voltage steps to 0 mV. Bottom: average current-voltage (I-V) relationship of $I_{Ca,L}$. * $P < 0.05$ versus HF control; † $P < 0.05$ versus control; ‡ $P < 0.05$ versus HF- $\omega 3$ (repeated measures ANOVA). **C**, Top: the Na^{+} - Ca^{2+} exchange current (I_{NCX}) was measured in Ca^{2+} -buffered conditions as the Ni^{2+} -sensitive current during a descending voltage ramp protocol. Middle: typical examples of I_{NCX} . Bottom: average I-V relationship of I_{NCX} .

both HF control and HF- $\omega 9$. The incidence of triggered APs was 17% and 14% in the HF control and HF- $\omega 9$ group, respectively, and significantly less in the control (0%) and HF- $\omega 3$ myocytes (7%).

Proteomics

A hypothesis free proteomic approach was followed to identify potential protein expression changes that could underlie the electrophysiological differences between the

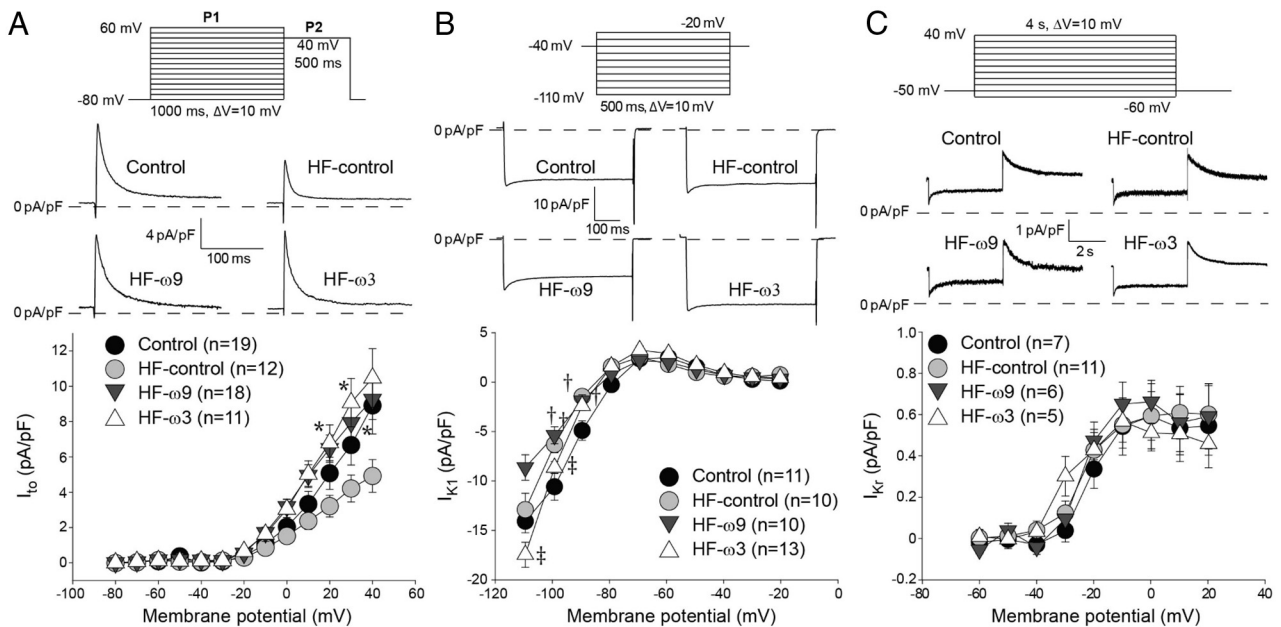


Figure 5. **A**, Top: 2-step protocol for transient outward K^{+} current (I_{to1}) measurement. Middle: typical examples of I_{to1} during voltage steps to 40 mV. Bottom: average current-voltage (I-V) relationship of I_{to1} . **B**, Top: protocol for inward rectifier K^{+} current (I_{K1}) measurement. Middle: typical examples of I_{K1} during voltage steps to -110 mV. Bottom: average I-V relationship of I_{K1} . * $P < 0.05$ versus HF control; † $P < 0.05$ versus control; ‡ $P < 0.05$ versus HF- $\omega 9$ (repeated measures ANOVA). **C**, Top: protocol for delayed rectifier K^{+} current (I_{Kr}) measurement. Middle: typical examples of I_{Kr} during voltage steps to 10 mV. I_{Kr} was defined as the tail current on stepping back to the holding potential. Bottom: average I-V relationship of I_{Kr} .

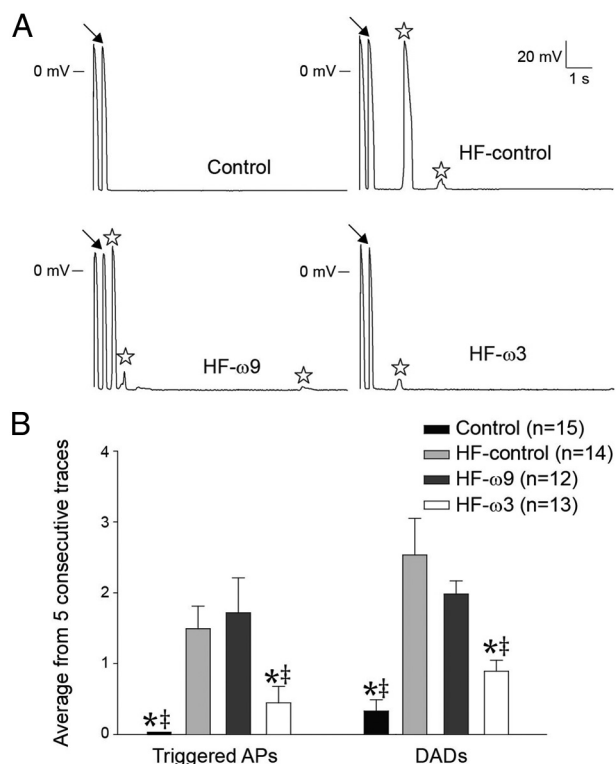


Figure 6. Fish oil prevents triggered activity. **A**, Representative examples of the tracing periods. Arrows indicate last paced action potential (AP). **B**, Data summary of the number of triggered APs and delayed after depolarizations. * $P < 0.05$ versus HF control and ‡ $P < 0.05$ versus HF- $\omega 9$ (1-way ANOVA).

HF- $\omega 9$ and HF- $\omega 3$ groups. In general, few significant differences were detected between HF- $\omega 9$, HF- $\omega 3$, and control groups. The main changes were represented by an increase of levels of cardiac albumin and very long-chain specific acyl-CoA dehydrogenase by HF- $\omega 9$, and an increase of levels of nicotinamide adenine dinucleotide dehydrogenase flavoprotein 1 and adenosine triphosphate synthase subunit by HF- $\omega 9$ and HF- $\omega 3$, compared with control (see online-only Data Supplement Figure I and Table VI).

Discussion

In this study we showed that long-term dietary supplementation with unsaturated FAs started before the induction of HF attenuated the development of cardiac hypertrophy and prevented progression toward HF. Supplementation with fish oil but not sunflower oil also prevented electric remodeling associated with HF, leading to reduced susceptibility of arrhythmias. The data support the notion that FA-enriched diets maintain myocardial FAO capacity and are associated with reduced progression of HF and arrhythmogenesis.

The reduced hypertrophy in the HF- $\omega 9$ group in itself makes the heart less vulnerable for re-entrant arrhythmias. However, the relatively long action potential, and increased diastolic Ca^{2+} and $I_{\text{Ca,L}}$, resulted in a similar number of arrhythmogenic triggers as in the HF control group. Thus, $\omega 3$ -FAs should be preferred over $\omega 9$ -FAs because these reduce both hypertrophy and triggered arrhythmias.

Unsaturated Fatty Acids Maintain Myocardial Fatty Acid Oxidation Capacity

Heart failure leads to a reduction in cardiac FAO capacity.¹ The increased CPT 1 activity and mRNA levels of the mitochondrial trifunctional protein in the HF- $\omega 3$ and HF- $\omega 9$ groups indicate that increased FA intake may counteract this. The hypothesis that FAs maintain myocardial FAO should be evaluated further by measuring the change in myocardial metabolism. Expression of both genes as well as many other genes involved in FAO are regulated by peroxisome proliferator-activated receptor, a transcription factor activated by FAs (including $\omega 3$ - and $\omega 9$ -FAs).²² Thus, our data confirm that unsaturated (fish oil) FAs suppress cardiomyocyte hypertrophy in vitro and in vivo, and we speculate that this was affected by activating proliferator-activated receptors.²²

The cardioprotective mechanisms of fish oils are diverse, and include antiarrhythmic, antiatherosclerotic, and anti-inflammatory effects.^{23,24} In addition, $\omega 3$ -FAs incorporate in the mitochondrial membranes of cardiac myocytes and change FAO and mitochondrial respiration.²⁵

Omega-3 and Omega-9 Fatty Acids Prevent Cardiac Hypertrophy

We found that both dietary $\omega 3$ - and $\omega 9$ -FAs prevented cardiac hypertrophy following combined volume and pressure overload. Epidemiological studies show that increased intake of $\omega 3$ -FAs is associated with reduced incidence of HF.²⁶ Similarly, fish oil diets in rats with pressure overload also prevented hypertrophy.²⁷ Although there is limited experimental and clinical evidence of the role of high fat diets in the pathogenesis of HF, both animal studies and human studies show that high unsaturated fat diets are not harmful for the heart and may even improve biomarkers of cardiovascular disease.²⁸

Omega-3 Fatty Acids Are Increased in Cardiac Tissue

In the HF- $\omega 3$ group, this diet increased myocardial levels of $\omega 3$ -FAs by $\approx 10\%$ at the expense of $\omega 6$ -FAs. In patients, cardiac $\omega 3$ -FAs may increase up to $\approx 6\%$ after fish oil supplementation for 1 month.²⁹ The similarity between our data and the levels obtained in man suggests that the effects observed in this study are relevant in human HF.

Note that the vaccenic acid (18:1n7) percentage is low, but highest in the HF- $\omega 3$ group, suggesting that this trans FA did not have any detrimental effect in our study, as suggested recently.³⁰

Unsaturated Fatty Acids Lower Triglycerides During Developing Heart Failure

Our data show that the HF- $\omega 3$ and HF- $\omega 9$ groups both had lower plasma triglycerides levels than the HF control group. Omega-3 FAs appear to be superior in lowering plasma triglycerides, as these were lower in the HF- $\omega 3$ group than in the HF- $\omega 9$ group. Also in humans, the Mediterranean diet, rich in monounsaturated fatty acids primarily from olives and olive oil, and fish oils markedly decreased triglyceride levels.³¹ Therefore, monounsaturated fatty acids not only have antihypertrophic effects, but also have favorable metabolic effects.

Dietary Fish Oil But Not Sunflower Oil Prevents Electric Remodeling in Heart Failure

In addition to a slowing effect on the progression of HF, the fish oil diet resulted in a reduced QTc interval on the surface ECG. This observation was supported in isolated myocytes of the HF- ω 3 group that displayed shorter APs. In all cardiomyopathy models, I_{to1} density is reduced.³² This was confirmed in our study in which we observed a \approx 50% decrease in I_{to} in the HF control group. The HF- ω 3 and the HF- ω 9 group did not display remodeling of I_{to} , which explains the shorter AP in the HF- ω 3 group. In the HF- ω 9 group, however, I_{CaL} was larger than in the other groups, explaining the longer APs and the higher diastolic Ca^{2+} levels compared with the HF- ω 3 group. In addition, increased Ca^{2+} influx through L-type Ca^{2+} channels by necessity leads, under steady state conditions, to increased efflux of Ca^{2+} through the Na^+-Ca^{2+} exchanger and to increased depolarizing I_{NCX} and AP prolongation. Because calcium handling was impaired in the HF- ω 9 group despite the normalization of SERCA2a mRNA, we cannot exclude that sarco/endoplasmic reticulum calcium ATPase function was compromised nevertheless.

Fish oil has disparate effects on ion currents, and both an increase and decrease in K^+ currents have been described.^{4,23,33} This appeared to depend on whether the FAs were applied to the cells or incorporated in the membranes.²³ Our study supports the idea that the effect of fish oil on cardiac arrhythmias not only depends on the mode of application, but also on the underlying cardiac pathology.

We followed a proteomics approach to identify potential protein expression changes between the HF- ω 9, HF- ω 3, and control groups. This failed to show major significant differences between groups, apart from an indication that oxidation of very long chain FAs and oxidative phosphorylation may be increased in HF- ω 9, and to a lesser extent HF- ω 3. Therefore, the observed electrophysiological differences between the HF- ω 9 and HF- ω 3 groups cannot be explained by regulation of visualized proteins in the 2D gel, albeit that our approach may not have been sufficiently sensitive to detect regulation of pore forming and accessory subunits for each individual ion channel that may have been affected by the dietary interventions.

Potential Significance and Limitations

Dietary fish oil supplements are recommended for patients after a myocardial infarction, as their cardioprotective efficacy has been demonstrated in various clinical trials on the Mediterranean diet.^{6,7,34} The result of the recent GISSI-HF,¹¹ however, showed a modest protection against cardiovascular death and hospitalization in patients with established HF.

We studied the effect of fish oil during the development of HF without interference of medical therapies. In this setting, fish oil supplementation prevented both structural and electric remodeling associated with HF. This may explain why fish oil supplementation causes a larger reduction in sudden death in patients at risk for HF (after acute myocardial infarction)⁷ than in patients with established HF,¹¹ even in the presence of optimal medical therapy. Indeed, increased fish oil consumption is reported to be associated with a reduced incidence of

HF.^{26,35} We would argue that the traditional diet-heart hypothesis may need to be adjusted after confirmation of our findings in man. The molecular mechanisms underlying these effects remain uncertain, although our data suggest that an increased capacity for myocardial FAO plays a role, as well as the alteration of ion channel kinetics.

We were unable to specify the phospholipid content of the sarcolemma in the 4 groups, and were limited by the paucity of suitable antibodies for validation of protein changes.

Conclusion

Dietary unsaturated FAs supplementation initiated before the development of HF reduces hypertrophy. In addition, fish oil FAs prevent HF-induced electric remodeling and triggered arrhythmias. Our study may explain why fish oil FAs are powerful antiarrhythmic drugs during the development of HF.

Acknowledgments

The authors thank Betty van der Struijs, Chantal Munts, and Jan Ruijter for excellent support.

Sources of Funding

This work was supported by the Netherlands Heart Foundation (grants 2003B079 and 2007B018), the SEAFOODplus program of the European Union (grant 506359), EU FP6 grant LSHM-CT-2005-018833/EUGeneHeart to M.v.B., and the Netherlands Organisation for Scientific Research (VIDI-grant No. 016.086.336 to S.M.H.).

Disclosures

None.

References

1. Van Bilsen M, Smeets PJH, Gilde AJ, Van der Vusse GJ. Metabolic remodelling of the failing heart: the cardiac burn-out syndrome? *Cardiovasc Res*. 2004;61:218–226.
2. Dávila-Román V, Vedala G, Herrero P, de las Fuentes L, Rogers JG, Kelly DP, Gropler RJ. Altered myocardial fatty acid and glucose metabolism in idiopathic dilated cardiomyopathy. *J Am Coll Cardiol*. 2002;40:271–277.
3. Janse MJ. Electrophysiological changes in heart failure and their relationship to arrhythmogenesis. *Cardiovasc Research*. 2004;61:208–217.
4. Verkerk AO, van Ginneken ACG, Berecki G, Den Ruijter HM, Schumacher CA, Veldkamp MW, Baartscheer A, Casini S, Opthof T, Hovenier R, Fiolet JW, Zock P, Coronel R. Incorporated sarcolemmal fish oil fatty acids shorten pig ventricular action potentials. *Cardiovasc Res*. 2006;70:509–520.
5. Den Ruijter HM, Berecki G, Verkerk AO, Bakker D, Baartscheer A, Schumacher CA, Belterman CNW, de Jonge N, Fiolet JWT, Brouwer IA, Coronel R. Acute administration of fish oil inhibits triggered activity in isolated myocytes from rabbits and patients with heart failure. *Circulation*. 2008;117:536–544.
6. Burr ML, Gilbert JF, Holliday RM, Elwood PC, Fehily AM, Rogers S, Sweetnam PM, Deadman NM. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: Diet And Reinfarction Trial (DART). *The Lancet*. 1989;334:757–761.
7. GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *The Lancet*. 1999;354:447–455.
8. Schrepf R, Limmert T, Claus Weber P, Theisen K, Sellmayer A. Immediate effects of n-3 fatty acid infusion on the induction of sustained ventricular tachycardia. *The Lancet*. 2004;363:1441–1442.
9. Den Ruijter HM, Verkerk AO, Berecki G, Bakker D, van Ginneken ACG, Coronel R. Dietary fish oil reduces the occurrence of early afterdepolarizations in pig ventricular myocytes. *J Mol Cell Cardiol*. 2006;41:914–917.
10. Billman GE, Hallaq H, Leaf A. Prevention of ischemia-induced ventricular fibrillation by omega-3 fatty acids. *Proc Natl Acad Sci*. 1994;91:4427–4430.

11. GISSI HF-investigators. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. *The Lancet*. 2008;372:1223–1230.
12. Baartscheer A, Schumacher CA, Belterman CNW, Coronel R, Fiolet JWT. SR calcium handling and calcium after-transients in a rabbit model of heart failure. *Cardiovasc Res*. 2003;58:99–108.
13. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem*. 1957;226:497–509.
14. de Groot JR, Schumacher CA, Verkerk AO, Baartscheer A, Fiolet JWT, Coronel R. Intrinsic heterogeneity in repolarization is increased in isolated failing rabbit cardiomyocytes during simulated ischemia. *Cardiovasc Res*. 2003;59:705–714.
15. Teunissen BEJ, Smeets PJH, Willemsen PHM, De Windt LJ, Van der Vusse GJ, Van Bilsen M. Activation of PPAR δ inhibits cardiac fibroblast proliferation and the transdifferentiation into myofibroblasts. *Cardiovasc Res*. 2007;75:519–529.
16. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paep A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology*. 2002;3:research0034.
17. Hellemans J, Mortier G, De Paep A, Speleman F, Vandesompele J. qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biology*. 2007;8:R19.
18. van Vlies N, Ruiter JPN, Doolaard M, Wanders RJA, Vaz FM. An improved enzyme assay for carnitine palmitoyl transferase I in fibroblasts using tandem mass spectrometry. *Mol Genet Metab*. 2007;90:24–29.
19. de Roos B, Duivenvoorden I, Rucklidge G, Reid M, Ross K, Lamers RJ, Voshol PJ, Havekes LM, Teusink B. Response of apolipoprotein E*3-Leiden transgenic mice to dietary fatty acids: combining liver proteomics with physiological data. *The FASEB Journal*. 2005;19:813–815.
20. Kerner J, Hoppel C. Fatty acid import into mitochondria. *Biochim Biophys Acta*. 2000;1486:1–17.
21. Wiegman RF, Verkerk AO, Belterman CN, van Veen TAB, Baartscheer A, Ophof T, Wilders R, de Bakker JMT, Coronel R. Larger cell size in rabbits with heart failure increases myocardial conduction velocity and QRS duration. *Circulation*. 2006;113:806–813.
22. Klier SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM, Lehmann JM. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ . *Proc Natl Acad Sci*. 1997;94:4318–4323.
23. Den Ruijter HM, Berecki G, Ophof T, Verkerk AO, Zock PL, Coronel R. Pro- and antiarrhythmic properties of a diet rich in fish oil. *Cardiovasc Res*. 2007;73:316–325.
24. Duda MK, O'Shea KM, Stanley WC. ω -3 polyunsaturated fatty acid supplementation for the treatment of heart failure: mechanisms and clinical potential. *Cardiovasc Res*. 2010;84:33–41.
25. O'Shea KM, Khairallah RJ, Sparagna GC, Xu W, Hecker PA, Robillard-Frayne I, Des Rosiers C, Kristian T, Murphy RC, Fiskum G, Stanley WC. Dietary ω -3 fatty acids alter cardiac mitochondrial phospholipid composition and delay Ca^{2+} -induced permeability transition. *JMCC*. 2009;47:819–827.
26. Mozaffarian D, Bryson CL, Lemaitre RN, Burke GL, Siscovick DS. Fish intake and risk of incident heart failure. *J Am Coll Cardiol*. 2005;45:2015–2021.
27. Duda MK, O'Shea KM, Tintin A, Xu W, Khairallah RJ, Barrows BR, Chess DJ, Azimzadeh AM, Harris WS, Sharov VG, Sabbah HN, Stanley WC. Fish oil, but not flaxseed oil, decreases inflammation and prevents pressure overload-induced cardiac dysfunction. *Cardiovasc Res*. 2009;81:319–327.
28. Chess DJ, Stanley WC. Role of diet and fuel overabundance in the development and progression of heart failure. *Cardiovasc Res*. 2008;79:169–278.
29. Metcalf RG, James MJ, Gibson RA, Edwards JRM, Stubberfield J, Stuklis R, Roberts-Thomson K, Young GD, Cleland LG. Effects of fish-oil supplementation on myocardial fatty acids in humans. *Am J Clin Nutr*. 2007;85:1222–1228.
30. Oie E, Ueland T, Dahl CP, Bohov P, Berge C, Yndestad A, Gullestad L, Aukrust P, Berge RK. Fatty acid composition in chronic heart failure: low circulating levels of eicosatetraenoic acid and high levels of vaccenic acid are associated with disease severity and mortality. *J Intern Med*. 2011;270:263–272.
31. Kastorini CM, Milionis HJ, Esposito K, Giugliano D, Goudevenos JA, Panagiotakos DB. The effect of Mediterranean diet on metabolic syndrome and its components: a meta-analysis of 50 studies and 534 906 individuals. *J Am Coll Cardiol*. 2011;57:1299–1313.
32. Verkerk AO, Baartscheer A, de Groot JR, Wilders R, Coronel R. Etiology-dependency of ionic remodeling in cardiomyopathic rabbits. *Int J Cardiol*. 2009;148:145–160.
33. Guizy M, Arias C, David M, Gonzalez T, Valenzuela C. ω -3 and ω -6 polyunsaturated fatty acids block HERG channels. *Am J Physiol Cell Physiol*. 2005;289:C1251–C1260.
34. Kris-Etherton PM, Harris WS, Appel LJ, for the Nutrition Committee. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation*. 2002;106:2747–2757.
35. Levitan EB, Wolk A, Mittleman MA. Fatty fish, marine ω -3 fatty acids and incidence of heart failure. *Eur J Clin Nutr*. 2010;64:587–594.

CLINICAL PERSPECTIVE

During heart failure, the cardiac metabolic substrate preference changes from fatty acid (FA) toward glucose. This change may contribute to progression of disease. We addressed whether a diet rich in unsaturated fatty acids limits progression toward HF. We fed rabbits with a diet rich in sunflower oil, fish oil, or control feed, and HF was induced by volume and pressure overload. Both the FA-supplemented groups had increased myocardial FA oxidation. The sunflower oil group had lower relative heart weight and had similar QTc interval as the HF control group. The fish oil group also displayed lower heart weight, but had shorter QTc intervals on the ECG. The shorter QTc was explained by action potential shortening in fish oil-fed animals and was caused by increased I_{to1} and I_{K1} , and resulted in reduced triggered arrhythmias in the fish oil group. Thus, dietary supplementation with unsaturated fatty acids prevents hypertrophy and HF, and fish oil supplementation additionally prevents HF-induced electric remodeling. These data may contribute to understanding the potential cardioprotective effects of Mediterranean diets in this setting. In addition, our data may shed light on why fish oil supplementation is beneficial in patients with a recent myocardial infarction, but not in patients with established HF.